

The role of genetic and genomic attributes in the success of polyploids

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In 1950, G. Ledyard Stebbins devoted two chapters of his book *Variation and Evolution in Plants* (Columbia Univ. Press, New York) to polyploidy, one on occurrence and nature and one on distribution and significance. Fifty years later, many of the questions Stebbins posed have not been answered, and many new questions have arisen. In this paper, we review some of the genetic attributes of polyploids that have been suggested to account for the tremendous success of polyploid plants. Based on a limited number of studies, we conclude: (i) Polyploids, both individuals and populations, generally maintain higher levels of heterozygosity than do their diploid progenitors. (ii) Polyploids exhibit less inbreeding depression than do their diploid parents and can therefore tolerate higher levels of selfing; polyploid ferns indeed have higher levels of selfing than do their diploid parents, but polyploid angiosperms do not differ in outcrossing rates from their diploid parents. (iii) Most polyploid species are polyphyletic, having formed recurrently from genetically different diploid parents. This mode of formation incorporates genetic diversity from multiple progenitor populations into the polyploid "species"; thus, genetic diversity in polyploid species is much higher than expected by models of polyploid formation involving a single origin. (iv) Genome rearrangement may be a common attribute of polyploids, based on evidence from genome *in situ* hybridization (GISH), restriction fragment length polymorphism (RFLP) analysis, and chromosome mapping. (v) Several groups of plants may be ancient polyploids, with large regions of homologous DNA. These duplicated genes and genomes can undergo divergent evolution and evolve new functions. These genetic and genomic attributes of polyploids may have both biochemical and ecological benefits that contribute to the success of polyploids in nature.

Polyploidy, the presence of more than two genomes per cell, is a significant mode of species formation in plants and was one of the topics closest to the heart of Ledyard Stebbins. In *Variation and Evolution in Plants*, Stebbins (1) devoted two chapters to polyploidy and addressed the following issues: the frequency, taxonomic distribution, and geographic distribution of polyploidy; the origins of polyploidy and factors promoting polyploidy; the direct effects of polyploidy; the polyploid complex; the success of polyploids in extreme habitats (including weeds); ancient polyploidy; and the role of polyploidy in the evolution and improvement of crops. He continued to explore these and other themes in his subsequent work, most notably in *Chromosomal Evolution in Plants* (2). In this paper we pay tribute to Ledyard, who was an inspiration and a friend, by exploring some of the questions that he asked about polyploids and by reviewing recent advances in the study of polyploidy.

Estimates of the frequency of polyploid angiosperm species range from $\approx 30\text{--}35\%$ (3) to as high as 80% (4); most estimates are near 50% (2, 5). Levels of polyploidy may be even higher in pteridophytes, with some estimates of polyploidy in ferns as high as 95% (5). Polyploids often occupy habitats different from those of their diploid parents, and have been proposed to be superior colonizers to diploids. Furthermore, most crop plants are of

polyploid origin, as noted by Stebbins (1). In contrast, although genome doubling has been reported from other major groups of eukaryotes (reviewed in ref. 6), it is not nearly as common in these groups as it is in plants.

The question often has arisen as to *why* polyploids are so common and so successful, and several possible explanations have been proposed. Stebbins (1) considered vegetative reproduction and the perennial habit to be important factors promoting the establishment of polyploids, along with an outcrossing mating system to allow for hybridization (between species, subspecies, races, populations, etc.) in the formation of the polyploid. Perhaps most important to Stebbins (1) was the availability of new ecological niches. Additional hypotheses for the success of polyploids include broader ecological amplitude of the polyploid relative to its diploid parents, better colonizing ability, higher selfing rates, and increased heterozygosity.

In fact, many aspects of the genetic systems of polyploids may contribute to the success of polyploid plants. These characteristics range from the molecular level to the population level and include increased heterozygosity, reduced inbreeding depression and an associated increase in selfing rates, increased genetic diversity through multiple formations of a polyploid species, genome rearrangements, and ancient polyploidy and gene silencing. But what role, if any, do these factors really play in the success of polyploids? In this paper, we will explore the evidence for the role of these genetic attributes in the evolutionary success of polyploid plant species.

Allo- Versus Autopolyploidy

We will distinguish among types of polyploids by using Stebbins' (1, 3) classification: Allopolyploids are those polyploids that have arisen through the processes of interspecific hybridization and chromosome doubling (not necessarily in that order), autopolyploids are those polyploids that have arisen from conspecific parents, and segmental allopolyploids are those that have arisen from parents with partially divergent chromosome arrangements such that some chromosomal regions are homologous between the parents and others are homoeologous; segmental allopolyploids will not be considered further in this paper. Allopolyploids are characterized by fixed (i.e., nonsegregating) heterozygosity, resulting from the combination of divergent parental genomes; bivalent formation occurs at meiosis, and disomic inheritance operates at each locus. Autopolyploids may exhibit multivalent formation at meiosis and are characterized by polysomic inheritance. Allopolyploids are considered much more prevalent in nature than are autopolyploids, but even a cursory glance at any flora (for example, see ref. 7) or list of plant

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Table 1. Genetic variation (mean values) in diploid (2n) and tetraploid (4n) populations (from ref. 29)

Species	P		H		A	
	2n	4n	2n	4n	2n	4n
<i>Tolmiea menziesii</i>	0.240	0.408	0.070	0.237	3.0	3.53
<i>Heuchera grossulariifolia</i>	0.238	0.311	0.058	0.159	1.35	1.55
<i>Heuchera micrantha</i>	0.240	0.383	0.074	0.151	1.41	1.64
<i>Dactylis glomerata</i>	0.70	0.80	0.17	0.43	1.51	2.36
<i>Turnera ulmifolia</i>						
var. <i>elegans</i>	0.459	0.653	0.11	0.42	2.20	2.56
var. <i>intermedia</i>	0.459	0.201	0.11	0.07	2.20	2.00

P, proportion of loci polymorphic; H, observed heterozygosity; A, mean number of alleles per locus.

chromosome numbers (for example, see ref. 8) will reveal multiple cytotypes within many species, even though these additional ploidal levels are not typically accorded species status. Thus, autopolyploids in nature likely are much more common than typically is recognized.

Increased Heterozygosity

Roose and Gottlieb (9) showed that allotetraploids in *Tragopogon* had fixed heterozygosity at isozyme loci, representing the combination of divergent genomes. In the allotetraploids *Tragopogon mirus* and *Tragopogon miscellus*, 33% and 43%, respectively, of the loci examined were duplicated. These values are typical of the levels of duplicated loci observed in many allotetraploid plants (10, 11). Of course, this value varies depending on the extent of allozyme divergence between the diploid progenitors: An allotetraploid derivative of two allozymically similar parents would display lower apparent levels of duplicated loci and fixed heterozygosity than would a derivative of more genetically divergent parents. However, even in those cases where there is no apparent allelic divergence between the parental genomes, the chromosomal segment is still duplicated; the possible fates of duplicated genes are reviewed by Wendel (6). All allopolyploid individuals are essentially heterozygous through nonsegregating, fixed heterozygosity.

Populations of autopolyploids are expected to maintain higher levels of heterozygosity than do their diploid progenitors (12, 13), and these higher heterozygosities can be attributed simply to polysomic inheritance (14). For example, assuming simple tetrasomic segregation, selfing of a heterozygous autotetraploid of genotype *aabb* is expected to produce progeny in the ratio of 1 *aaaa*:34 heterozygotes (of various genotypes):1 *bbbb*, a huge increase over expectations for a diploid with disomic inheritance (i.e., 1 *aa*:2 *ab*:1 *bb*).

Empirical studies have demonstrated that autotetraploids with tetrasomic inheritance do indeed have higher levels of heterozygosity than do their diploid parents (15). For example, *Tolmiea menziesii*, which occurs along the Pacific Coast of North America from central California to southeastern Alaska, comprises diploid populations, which are distributed in the southern portion of the range, and tetraploid populations, which occupy the northern portion. Various measures of genetic diversity were compared in natural populations of the two cytotypes (15). At seven polymorphic isozyme loci, a substantially larger number of tetraploid individuals was heterozygous as compared with diploid individuals (15), and comparisons of diploid and autopolyploid populations in other species show the same pattern (Table 1). Many other polyploids also exhibit polysomic inheritance (Table 2); consequently, these polyploids likely also maintain higher levels of heterozygosity than do their diploid parents, simply because of their mode of inheritance.

Table 2. Examples of polysomic inheritance (from ref. 29)

Species	Inheritance	Evidence
<i>Allium nevii</i>	Tetrasomic	Isozymes
<i>Chrysanthemum morifolium</i>	Hexasomic	Morphology
<i>Daelylis glomerata</i>	Tetrasomic	Isozymes
<i>Dahlia variabilis</i>	Tetrasomic	Morphology
<i>Haplopappus spinulosus</i>	Tetrasomic	Isozymes
<i>Heuchera grossulariifolia</i>	Tetrasomic	Isozymes
<i>Heuchera micrantha</i>	Tetrasomic	Isozymes
<i>Lotus corniculatus</i>	Tetrasomic	Cyanogenic markers, isozymes
<i>Lythrum salicaria</i>	Tetrasomic	Morphology
<i>Maclura pomifera</i>	Tetrasomic	Isozymes
<i>Medicago falcata</i>	Tetrasomic	Morphology, isozymes
<i>Medicago sativa</i>	Tetrasomic	Morphology, isozymes
<i>Pachycereus pringlei</i>	Tetrasomic	Isozymes
<i>Phleum pratense</i>	Hexasomic	Morphology
<i>Solanum tuberosum</i>	Tetrasomic	Morphology, isozymes
<i>Tolmiea menziesii</i>	Tetrasomic	Isozymes
<i>Turnera ulmifolia</i>		
var. <i>elegans</i>	Tetrasomic	Isozymes
var. <i>intermedia</i>	Tetrasomic	Morphology, isozymes
<i>Vaccinium corymbosum</i>	Tetrasomic	Isozymes

Outcrossing Rates in Polyploids and Their Diploid Progenitors

Some aspects of polyploid success have been attributed to improved colonizing ability, which may involve higher selfing rates than those of the diploid parents. We will present both theory on why we might expect increased selfing in polyploids and empirical data for selected ferns and angiosperms.

Theoretical models predict reduced inbreeding depression in polyploids relative to their diploid parents, because of the buffering effect of additional genomes: Deleterious alleles are masked by the extra genomes (2, 16, 17). Both allopolyploids and autopolyploids are expected to have reduced inbreeding depression (ref. 18, except under their overdominance model, and ref. 19), and the magnitude of inbreeding depression is negatively correlated with selfing rates in diploid angiosperms and gymnosperms (20). Unfortunately, few studies have addressed levels of inbreeding depression in polyploids empirically; most of the available data come from ferns.

Inbreeding depression in ferns (often referred to as genetic load in these studies) has been estimated by taking advantage of the life cycle that involves a free-living, haploid gametophyte generation that can, in most cases, self-fertilize to produce a completely homozygous diploid sporophyte. These studies have involved culturing gametophytes in isolation, in sib pairs, and in non-sib pairs. The number and survival of sporophytes resulting from these treatments are recorded, and these data can be used to estimate inbreeding depression, outcrossing depression, and the number of lethal equivalents per genome. If a greater number of normal sporophytes is produced by non-sib pairs of gametophytes than by either sib pairs or isolated gametophytes, then the population or species is considered to exhibit inbreeding depression.

Masuyama and Watano (21) reported two studies of inbreeding depression in diploid and tetraploid pairs of ferns. In *Phegopteris*, 30–60% of selfed gametophytes of the diploid race formed sporophytes, and nearly 100% of all selfed gametophytes of the tetraploid race formed sporophytes. In *Lepisorus*, only 4% of selfed gametophytes of the diploid race produced normal sporophytes, whereas 98–100% of the gametophytes of the tetraploid race formed sporophytes. These data were interpreted as evidence for reduced inbreeding depression in the tetraploid, with the lower inbreeding depression allowing for increased selfing rates.

There are few estimates of selfing rates in polyploid fern species, largely because polyploid fern populations often lack sufficient levels of segregating allozyme markers; however, selfing rates have been estimated in a few diploid-tetraploid pairs. In *Polystichum*, the allotetraploid *Polystichum californicum* has a selfing rate of 0.236, whereas selfing rates in the two diploid progenitors, *Polystichum dudleyi* and *Polystichum imbricans*, are only 2–3% (22). In tetraploid *Pteris dispar*, selfing rates are 0.84, much higher than the rate of 0.01 estimated for the diploid race (21). Limited evidence for ferns suggests reduced inbreeding depression and higher selfing rates in tetraploids than in diploids.

Comparisons of outcrossing rates and levels of inbreeding depression in diploid and polyploid angiosperms also are rare. Both outcrossing rates and inbreeding depression have been estimated for diploid and autotetraploid populations of *Epilobium angustifolium* (23, 24). Outcrossing rates in the two cytotypes were very similar; values (after correcting for inbreeding depression in the diploid) are 0.45 and 0.43 for diploids and tetraploids, respectively. However, the tetraploids have substantially lower inbreeding depression (0.95 for diploids versus 0.67 for tetraploids), as expected from population genetic theory.

Outcrossing rates also have been estimated in diploid and allotetraploid species of *Tragopogon* (25, 26). Outcrossing rates in the allotetraploid *T. mirus* (0.381 and 0.456 for two populations) were higher than those found in the diploid parent *Tragopogon dubius* (0.068 and 0.242), although significantly higher than only one of the two populations; the other parent, *Tragopogon porrifolius* (27, 28), lacked segregating allozyme variation from which to estimate outcrossing rates. This pattern is exactly the opposite of that predicted by population genetic theory, and one explanation offered to explain it is that rates of outcrossing were underestimated, particularly in *T. dubius*, because of limited polymorphic loci in all populations. To account for this possibility, outcrossing rates were estimated in *T. mirus* and *T. dubius* from artificial arrays constructed to maximize the chances of detecting an outcrossing event if one had occurred. Outcrossing rates ranged from 0 to >1 for diploid and tetraploid families, and the mean values were quite similar (0.696 and 0.633, respectively, for *T. mirus* and *T. dubius*) and higher than those estimated for natural populations, suggesting that some outcrossing events in both species, and especially the diploid *T. dubius*, had gone undetected (26). If the outcrossing rates estimated from the artificial arrays are more accurate than are those from natural populations, the discrepancy between predictions and results may be attributable to the recent ancestry of *T. mirus* (most likely post-1928; refs. 27 and 28) and to the limited time for the mating systems to have diverged.

The Genetic Implications of Recurrent Polyploid Formation

The application of isozyme analysis and DNA techniques to the study of polyploid ancestry dramatically altered our view of polyploid origins. Although morphological or cytological differences among populations of a few polyploid species suggested evidence of repeated polyploid formation (see example in ref. 27), most polyploid species, until recently, were considered to have had a unique origin. Nearly all polyploid species of plants that have been examined with molecular markers have been shown to be polyphyletic, having arisen multiple times from the same diploid species (reviewed in refs. 29–31). Polyphyletic polyploid species have been reported for mosses (32), ferns (33–35), and many angiosperms (29–31), and include both autopolyploids [e.g., *Heuchera grossulariifolia* (36–38) and *Heuchera micrantha* (39,40)] and allopolyploids (29–31). Recurrent formation of a polyploid species has implications for the taxonomy of polyploids, our understanding of the ease with which and rate at which polyploidization can occur, and, most relevant here, the genetic diversity of polyploid “species.” In this section, we will address (i) the proportion of polyploid plant

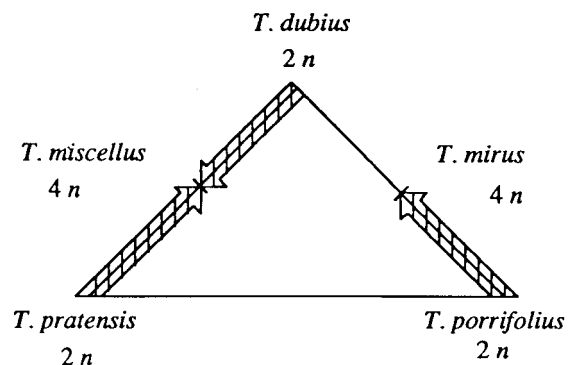


Fig. 1. Parentage and reciprocal origins of tetraploid species of *Tragopogon* in North America. Hatched lines indicate diploid(s) contributing chloroplast to the tetraploids.

species that are known to have formed recurrently, (ii) the extent of recurrent formations within a species, and (iii) the genetic and evolutionary significance of these multiple origins.

Most polyploid species examined to date have shown evidence of recurrent formation (29–31). Remarkably, these independent origins have been identified even though sampling strategies typically were not designed to investigate multiple origins but rather to test hypotheses of diploid parentage. In many cases, as few as two or three populations of a polyploid species were sampled; the genetic distinctness of these populations, coupled with additivity of diploid genotypes, strongly supported interpretations of recurrent formation. All available data suggest that nearly all polyploid species analyzed comprise multiple lineages of independent formation.

How many such lineages are present within a given polyploid species? Few studies have explicitly addressed this question. Two allotetraploid species of *Tragopogon*, *T. mirus* and *T. miscellus*, arose within the past century in the Palouse region of eastern Washington and adjacent Idaho from diploid progenitors that had been introduced to the region from Europe in the early 1900s (ref. 27; Fig. 1). During the past several decades, the ancestries of these two tetraploids have been investigated by using nearly every technique that has become available (41), and Ownbey's (27) interpretations have been confirmed.

Early morphological and cytological data (27, 42, 43) suggested multiple origins of each species, two of *T. miscellus* and three of *T. mirus*, in different locations on the Palouse. Recent isozyme and DNA analyses have supported Ownbey's (27) original hypotheses of recurrent origin and have identified additional lineages of independent formation (9, 28, 44, 45). For example, based on the geographic distribution of isozyme multilocus genotypes, chloroplast DNA haplotypes, and rDNA markers, estimates of the number of lineages in *T. mirus* ranged from 4 to 9 (with an extinct population of independent origin, based on flavonoid markers; ref. 46), and the number in *T. miscellus* ranged from 2 to 21 (28). However, several populations of *T. mirus* in different locations had the same isozyme multilocus genotype, chloroplast DNA haplotype, and rDNA repeat, and, in many cases, they co-occurred with the diploid progenitor species, *T. dubius* and *T. porrifolius*; the same was true of *T. miscellus*, which co-occurred in at least some locations with both of its progenitors, *T. dubius* and *Tragopogon pratensis*. It was possible that these separate locations represented independent sites of polyploid formation from genetically identical (based on the markers at hand) diploids. However, this hypothesis could not be tested without the use of more sensitive markers.

Cook *et al.* (41) used random amplified polymorphic DNA (RAPD) markers to test the hypothesis that isozymically identical populations of *T. mirus* having the same chloroplast DNA haplotype and rDNA repeat were of separate origin and that

“identical” populations of *T. miscellus* also were of separate origin. For *T. mirus*, five populations with isozyme multilocus genotype 1 (28) and two populations with isozyme genotype 2 (28) were sampled. Each population had a unique RAPD profile (and, in fact, two populations were polymorphic), suggesting that each population may have had a separate origin. Taken with other data, *T. mirus* may represent a collection of as many as 11 lineages (41). RAPD data for three populations of isozyme genotype 1 (28) of *T. miscellus* demonstrated that all three were distinct and possibly of separate origin, raising the number of genetically distinct populations of *T. miscellus* to five (41).

The *Tragopogon* tetraploids represent remarkable cases of recurrent formation on a small geographic scale and in a short period, perhaps the last 70 years. Other polyploid species, if examined in sufficient detail, may be similarly grossly polyphyletic. Furthermore, recurrent formation of *T. mirus* and *T. miscellus* also has occurred on a broader geographic scale. Both species have been reported from Flagstaff, AZ (47), and *T. miscellus* has been reported from Gardiner, MT, and Sheridan, WY (M. Ownbey, unpublished notes cited in ref. 9; Sheridan site confirmed by P.S.S. and D.E.S. in 1997; *T. miscellus* not observed in Gardiner in 1997).

Although such polyphyly calls into question the meaning of the term “polyploid species,” the biological implications of recurrent polyploidizations from the same diploid progenitor species are indeed intriguing. Such multiple formations may play a significant role in shaping the genetic structure of polyploid species, as they are currently recognized. The concept of recurrent formations forces us to consider polyploid species not as genetically uniform, as previous models of polyploid formation imply, but as genetically variable. In fact, multiple formations may represent a significant source of genetic diversity in polyploid species, as a polyploid species may comprise multiple, genetically different lineages. Finally, crossing between individuals of separate origin will break down the distinctions among lineages and may produce novel genotypes through recombination.

The long-term evolutionary significance of recurrent polyploid formations is unclear; however, a host of specific questions can be addressed. For example, do plants of different origins have distinct evolutionary potentials? Does recurrent formation lead to different locally adapted genotypes? How extensive is gene flow between populations of independent origin, and to what extent does gene flow contribute to the genetic diversity of populations? How frequently are new genotypes produced through recombination?

Genome Rearrangements in Polyploids

Another possible source of genetic novelty in polyploids is genome rearrangements. Evidence for chromosomal changes has been obtained through a number of techniques, including genome *in situ* hybridization (GISH), analysis of restriction fragment length polymorphism (RFLP) loci, and chromosome mapping. Among the earliest studies reporting widespread genomic changes in tetraploids relative to their diploid progenitors is an analysis of tobacco genome structure using GISH (reviewed in ref. 48). Tobacco (*Nicotiana tabacum*) is an allotetraploid whose parents are *Nicotiana glauca* and a T-genome diploid from section *Tomentosae* (48). GISH clearly revealed numerous chromosomal rearrangements. In fact, nine intergenomic translocations have occurred within the genome of tobacco, that is, translocations between the chromosomes donated by *N. sylvestris* and the T-genome parent. Most of the chromosomes of tobacco are therefore mosaics, composed of regions of both parental chromosome sets.

In *Brassica*, there is evidence that such genome rearrangements may occur very soon after the formation of the tetraploid. Song *et al.* (49) produced artificial tetraploids resulting from interspecific crosses between *Brassica rapa* and *Brassica nigra*

Table 3. Angiosperm families with high chromosome numbers, suggested to be of ancient polyploid origin (from ref. 1)

Basal angiosperms	Chromosome number, <i>n</i>
Family	
Illiciaceae	14
Schisandraceae	14
Lauraceae	12
Calycanthaceae	12
Magnoliaceae	19
Eudicot families	
Trochodendraceae	19
Platanaceae	21
Cercidiphyllaceae	19
Salicaceae	19
Hippocastanaceae	19

and between *B. rapa* and *Brassica oleracea*. They compared genome structure in the F₃ derivatives of these crosses with their F₂ ancestors and found genetic divergence in these few generations, with distances as high as almost 10%. In addition, Song *et al.* (49) found evidence of cytoplasmic–nuclear interactions—the maternal genotype had definite control over aspects of the nuclear genome. They concluded that a possible result of polyploid formation is the production of novel genotypes. Furthermore, extensive genetic change can occur in the early generations after polyploid formation and may therefore be important in the formation of a functional polyploid. Chromosome mapping of diploid *Brassica* and comparison with the map of *Arabidopsis thaliana* suggest that the diploid species of *Brassica* (*n* = 9) may actually be ancient hexaploids (ref. 50, but see ref. 51 for a different interpretation).

Such intergenomic translocations are not limited to tobacco and *Brassica*. Instead, extensive chromosomal changes have been reported in a number of other polyploids, including maize, oats, and soybeans. Such intergenomic translocations may be mediated by transposable elements (52) and may be an important source of genetic novelty in polyploids (see also ref. 6). Furthermore, cytoplasmic–nuclear interactions may be important in the establishment of a fertile polyploid (reviewed in ref. 48).

Ancient Polyploidy and Gene Silencing

Basal Angiosperms. Estimates of ancient polyploidy generally have relied on chromosome number alone; Stebbins (1), for example, viewed those plants with a base chromosome number of *n* = 12 or higher to be polyploid, and others (5, 53, 54) used similar criteria. Based on this criterion, a large number of angiosperm families, most of which trace their roots far back into angiosperm phylogeny, are considered to be the products of ancient polyploid events whose diploid ancestors are now extinct. For example, the Illiciales have *n* = 14, and both the Lauraceae and Calycanthaceae of Laurales have a base number of *n* = 12. The lowest chromosome number in the Magnoliaceae is *n* = 19, and the family exhibits a range of numbers that are multiples of this base number. Some early eudicots, such as *Trochodendron* and *Tetracentron* (with *n* = 19) and *Platanus* (with *n* = 21), also have high chromosome numbers. Some families of more recent origin [e.g., Salicaceae (willows and poplars), Hippocastanaceae (horse chestnuts and buckeyes), *Fraxinus* (ashes) and other Oleaceae, and *Tilia* (linden and basswood)] also are considered ancient polyploids. Some families of possible ancient polyploid origin, along with their chromosome numbers, are listed in Table 3, and the phylogenetic distribution of these families (on portions of the tree of refs. 55 and 56) is shown in Fig. 2. Stebbins (1, 2) also suggested that the ancestral base chromosome number for angiosperms is *x* = 6, 7, or 8; other, later authors (5, 54, 57, 58) have concurred. Reconstruction of chromosomal evolution

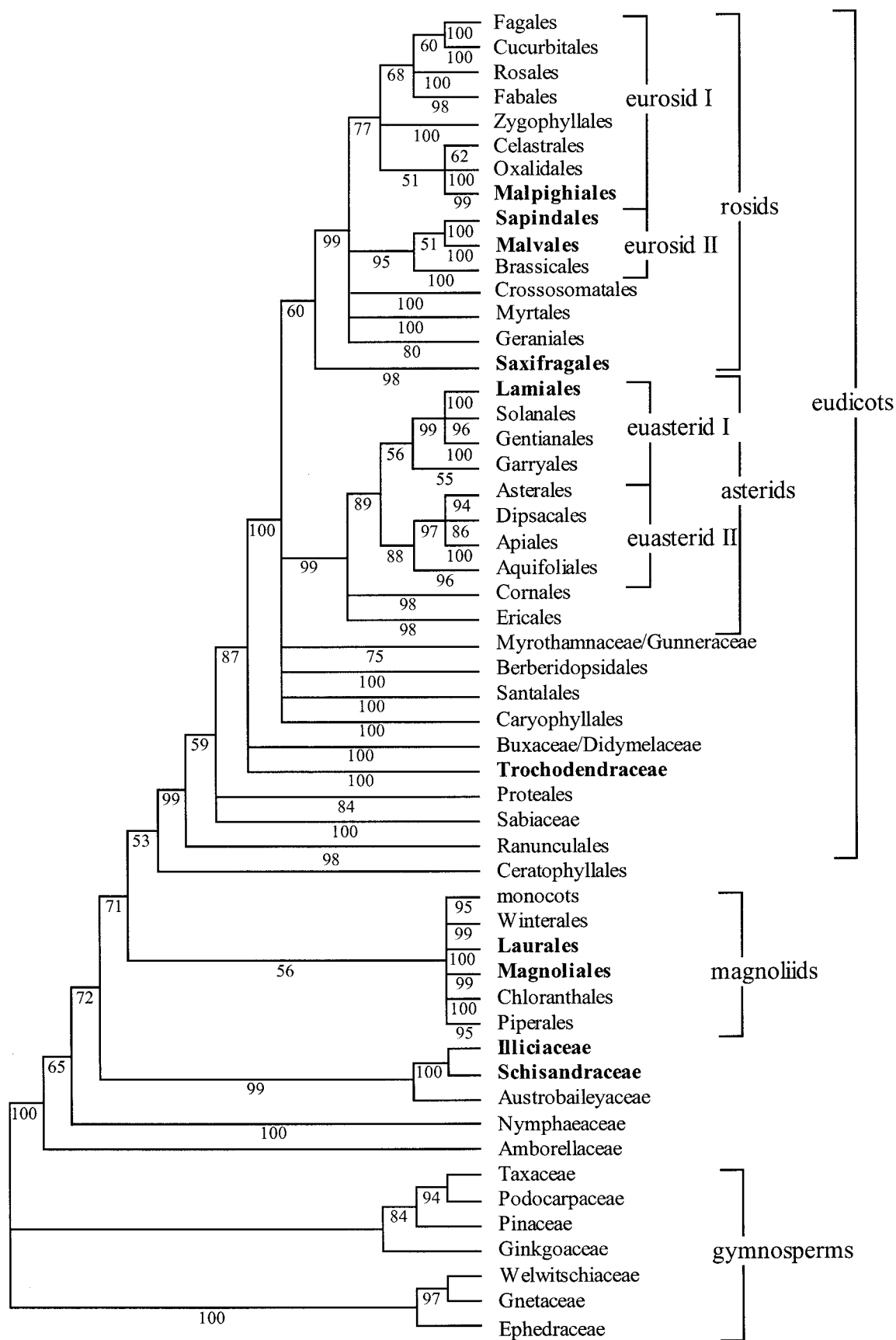


Fig. 2. Summary phylogenetic tree of angiosperms based on analyses of *rbcl*, *atpB*, and 18S rDNA sequences; redrawn from ref. 55. Clades with families of putative ancient polyploid origin are indicated in bold. Numbers below branches are jackknife support values.

across the angiosperms is partially consistent with Stebbins' hypothesis. Although the high chromosome numbers of the basal angiosperm groups make it difficult to infer base chromosome numbers for those groups of angiosperms and therefore for angiosperms as a whole, our reconstructions show an ancestral number of $x = 8$ for the eudicots (D.E.S., unpublished data), that is, the large clade that makes up 75% of angiosperm species. Identifying the ancestral number for all angiosperms will require teasing apart the base numbers of the ancient polyploid groups and will require further work.

Most, if not all, angiosperms may have experienced one or more cycles of genome doubling (6), and these hypotheses of ancient polyploidy have several implications for the genetics, genomics, and evolutionary biology of these plants. First, if they are indeed polyploids, then these plants should exhibit extra copies of their genes above the level that one would expect for diploid plants (10, 11). Analyses of enzyme expression indicate that multiple enzymes are indeed expressed in putatively paleopolyploid angiosperm families, such as those listed in Table 3 (59); issues of the regulation of duplicated genes are discussed by Wendel (6). Second, some copies of these multiple genes might be expected to be silenced, particularly in the more ancient families (see *Gene Silencing* below). Third, reorganization of the original polyploid genome might have led to a novel genomic arrangement and perhaps to novel phenotypes. Finally, given that all members of a family have chromosome numbers that are multiples of a single lower number, it appears that, after polyploidization, diversification continued at the new polyploid level, with subsequent episodes of polyploidy superimposed on this initial polyploid level. This pattern of divergent speciation at the polyploid level contradicts the view of polyploids as evolutionary dead-ends.

Homosporous Pteridophytes. Homosporous pteridophytes are those ferns (including *Psilotum* and *Tmesipteris*; refs. 60–62), lycophytes, and *Equisetum* with a homosporous life cycle; all of these groups are the descendants of ancient plant lineages that extend back to the Devonian Period (63). The mean gametic chromosome number for homosporous pteridophytes is $n = 57$; for angiosperms, it is $n = 16$ (64). Despite their high chromosome numbers, however, homosporous pteridophytes exhibit diploid gene expression at isozyme loci (65–68). At least two possible explanations can explain this paradox of high chromosome numbers and genetic diploidy. First, these plants are ancient polyploids that have undergone extensive gene silencing to produce genetic diploids, and second, they may have achieved high chromosome numbers through another mechanism, such as chromosomal fission.

Gene Silencing. Genes duplicated through polyploidy have several possible fates: retention of both copies as functional genes, acquisition of new function by one copy, and gene silencing (6). Several models of genome evolution, in which a polyploid genome gradually will undergo gene silencing and return to a diploid condition, have been presented (69, 70). Unfortunately, little empirical evidence is available to support or to refute these models.

Potential examples occur in the homosporous pteridophytes. Data for the ferns *Polystichum munitum* ($n = 41$) and *Ceratopteris richardii* ($n = 39$) may address these alternatives. Pichersky *et al.* (71) studied the genes for the chlorophyll *a/b* binding proteins in *P. munitum*. These proteins are important in photosynthesis and are encoded by a small multigene family (71). *P. munitum* exhibits diploid isozyme expression (22, 35, 72). If this species is of ancient polyploid origin but has since undergone substantial gene silencing, then pseudogenes should be detectable in the genome. Five clones of the *CAB* genes were analyzed by Pichersky *et al.* Three of the five clones were structurally

nonfunctional, a fourth clone had a structurally intact sequence but was nonfunctional at the sequence level, and a fifth clone was a functional sequence. Possible explanations for these results (71) are (i) amplification of nonfunctional sequences in the genome of *P. munitum*, regardless of the ploidy of *P. munitum*, (ii) *P. munitum* is diploid with a large number of mutant *CAB* genes, and (iii) *P. munitum* is polyploid, with silencing of multiple genes that are present because of ancient polyploidy. In *C. richardii*, cDNA clones hybridized to multiple fragments on genomic DNA blots, suggesting that 50% or more of these expressed sequences were present in multiple copies in this fern genome (73). In contrast, a similar experiment with *A. thaliana* detected only 15% duplicated fragments (74). Further characterization of the hybridizing fragments of the genome is necessary to document that they are in fact duplicated sequences. However, this evidence for multiple hybridizing fragments in *C. richardii*, along with the *CAB* gene data for *P. munitum*, suggests that the genomes of homosporous ferns may in fact be anciently polyploid.

Gene silencing remains an underinvestigated area of polyploid research. If it occurs as described in models of wholesale diploidization of the polyploid genome (70), what are the mechanisms and at what rate does such silencing occur? Or does silencing occur gradually, essentially one locus at a time? Many unanswered questions remain.

Conclusions

Leitch and Bennett (48) have suggested that the evolutionary potential of a polyploid depends on a number of factors associated with the formation of the polyploid and with genetic divergence between the parents; unfortunately, the factors involved in the origin and establishment of polyploids in nature are largely unknown (75). The success of a polyploid may depend, in part, on the parental origin of particular DNA sequences—is the sequence maternal or paternal and does it interact favorably with the organellar genomes? The type of sequence under study also may be important: is it coding or noncoding DNA, is it telomeric or centric in origin, and is it located near heterochromatin? Finally, what is the level of genetic differentiation between the parents?

Although unreduced gamete production and even polyploid formation may be quite common in many groups of plants (75), there are many obstacles to establishment of a polyploid population. Minority cytotype exclusion (76–78) may be particularly important in newly formed outcrossing polyploids where there are few potential mates unless there is substantial assortative mating (79); when only one or a few polyploid individuals emerge within a population of diploids, outcrossing polyploid individuals may spend most of their gametes in sterile or partially sterile matings with their diploid parents. The apparent success of polyploids is biased toward those species that have overcome the barrier(s) to establishment, and this success may ultimately derive from a number of the genetic attributes of the polyploids. Polyploids have increased heterozygosity, an attribute that may be beneficial (80, 81). Polyploids also harbor higher levels of genetic and genomic diversity than was anticipated, with recurrent formation from genetically divergent diploid parents and possibly genome rearrangements contributing genetic diversity. This genetic diversity results in greater biochemical diversity, which also may be beneficial to the polyploid (82). Finally, these genetic attributes may have ecological consequences. For example, if polyploids have lower inbreeding depression and are more highly selfing, they may be better colonizers, explaining the prevalence of polyploids on the list of the world's worst weeds. Polyploids may have broader ecological amplitudes than do their diploid progenitors because of their increased genetic and biochemical diversity (82). Polyploids may experience new interactions with other species, such as pollinators (83, 84).

What are some of the future directions we see for research on the genetic attributes of polyploids? The general mode of formation of polyploids remains unknown; research into the factors that produce unreduced gametes and bring them together certainly is warranted. Additional studies, both theoretical and empirical, are needed to address expectations of inbreeding depression and outcrossing rates. Furthermore, the levels of gene flow among populations, especially those populations of separate origin, are unknown. Regarding genome rearrangements, how extensive are they within an individual or race? How widespread are they among species? How quickly do such rearrangements occur? Do populations of separate origin exhibit the same or different rearrangements? Finally, are basal angiosperms and homosporous pteridophytes

with high chromosome numbers of ancient polyploid origin? If so, what can we learn about gene silencing from these plants? How extensive has gene silencing been, and is there evidence for the cooption of duplicated genes for new function? The study of polyploidy is a dynamic and open area of research, ranging from molecular genetic comparisons to population genetics, with important implications for the biology and evolution of the majority of plant species.

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